Meta-analysis of QTLs involved in rice root development using a QTL database

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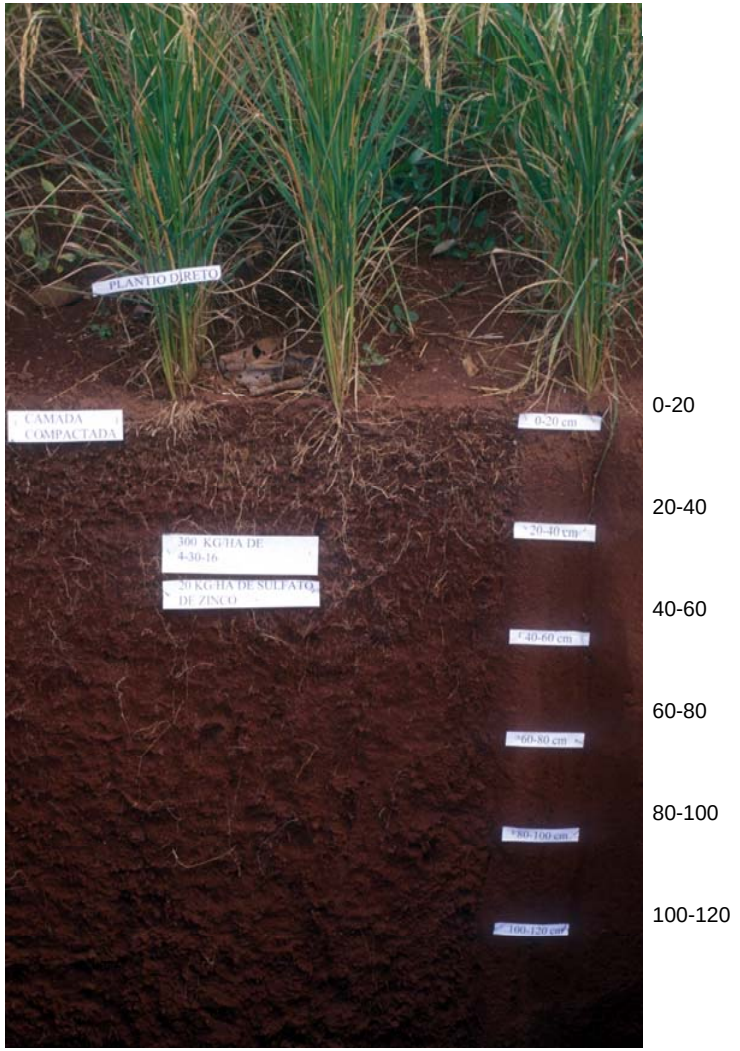
Importance of studying root development

- No "universal" trait to confer drought resistance
- Avoidance strategy = reasonable option in many agronomical situations
- Root depth = most consensual of all traits contributing to drought avoidance (at least for upland conditions)
- Essential role of root system for the realization of yield potential in favorable environment :
  - Anchoring
  - Capture of mineral and water resources
  - Early vigor and competitiveness with weeds
  - Site of hormone biosynthesis

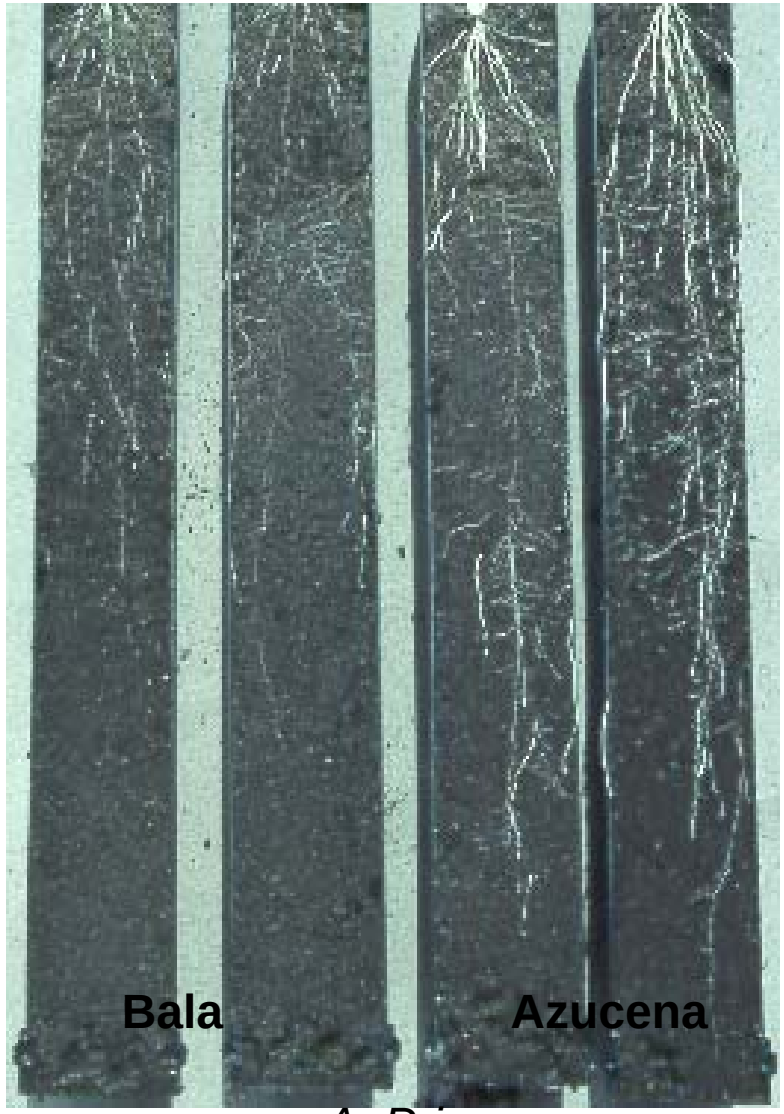


Brazil, 1994

Components of root system development

- **Constitutive component:** Intrinsic pathways determine the characteristic architecture of the root system in a given plant species or variety

- **Adaptive component**: Response pathways co-ordinate environmental cues with development by modulating intrinsic pathways (e.g. N, P, water stress, submergence..)



A. Price

Main descriptors of root development

- Maximum root length
  - Colonization of soil profile
  - Baseline for access to nutrient and water resources
- Root number
  - Intensity of soil colonization
  - Aptitude to exploit soil resources within the baseline
- Root thickness
  - Root specific length & contact surface with the soil
  - Aptitude to exploit soil resources
  - Response to soil physical condition
- Root ramification
  - Root length density
  - Aptitude to exploit soil resources within the baseline
- Allometric rations (R/S, RN/TN, DR/S, …) describing co-ordination with aerial growth and development

Genetic diversity for root development in rice

- Variation in root number, diameter, depth, branching, vertical density distribution, root to shoot ratio, water extraction, root penetration.
- The pattern of variation reflects the genetic structuration of *Oryza sativa*

G1 (*indica*)	G2	G3, G4, G5	G6 (*japonica*)
Thin, highly branched, Superficial, narrow vessels, low R/S ratio	Intermediate roots distribution, as G6, but thin roots	Same root thickness and root distribution as G1	Thick, less branched, long roots, large root to shoot ratio

- Diversity between groups rather than within, with the exception of groups 2 and 6 that show a sub-structure linked to ecosystem adaptation e.g. temperate versus tropical types in group 6 or boro versus aus in group 2 (Courtois et al, 1997; Lafitte et al, 2001)
- *O. glaberrima* does not appear to be a better source of alleles than tropical japonicas for root mass at depth. Same remark for wild species (Liu et al, 2004)

Genetic basis of root development in rice

- Large amount information available
  - Large number of QTL mapping papers reported for root development
  - Many mapping populations used
  - Large number of root traits analysed
  - Many growth conditions
  - Very large number of QTLs mapped
- Some successful MAS allredy realised and some cloning works ongoing
- Need for the integration of existing information
  - Framework for easy access to the whole root QTL data
  - Combining data from different studies to detect consensus QTL

Easy access to data: Rice root QTL database

- Development of a database with systematic description of QTL studies and QTL info
- Facilitate comparison between studies by projecting each QTL on a reference map : physical position of the markers flanking the QTL on the pseudochromosomes
- Making these information available through a web site (soon in http://tropgenedb.cirad.fr/)

Twelve mapping populations

Cross	Cross type	Population type	Reference of the studies
IR58821-23-B / IR52561-UBN-1-	*Indica / indica*	RILsF7	1
Zhenshan97 / Minghui63	*Indica / indica*	RILsF10	18
Bala/Azucena	*Indica / japonica*	RILsF6	9, 11, 12, 13
Co39 / Moroberekan	*Indica / japonica*	RILsF7	2, 14
IR1552 / Azucena	*Indica / japonica*	RILsF10	23
IR64 / Azucena	*Indica/japonica*	DH	4, 16, 19, 24
Zhenshan97 / IRAT109	*Indica / japonica*	RILsF9	20
Labelle / Black Gora	*Japonica* / Aus	F2:3	15
CT9993-5-10-1-M / IR62266-42-6-2	*Japonica / indica*	DH	6, 7, 10, 17, 21
IAC165 / Co39	*Japonica / indica*	RILsF7	3
Akihikari / IRAT 109	*Japonica / japonica*	BC1F4	5
IRAT109 / Yuefu	*Japonica / japonica*	DH	8

29 root traits mapped

Trait name	Symbol	Nbr populations	Nbr QTLs	Reference of the studies
Root **number**	RN	6	41	1, 4, 8, 12, 14, 23, 24
Deep root **number**	DRN	1	11	13
Lateral root **number**	LRN	1	3	23
Penetrated root **number**	PRN	4	27	1, 12, 14, 24
Seminal root **length**	SRL	1	7	23
Maximum root **length**	MRL	10	98	3, 4, 5, 6, 7, 9, 11, 13, 15, 18, 19, 20, 22
Lateral root **length**	LRL	1	5	23
Penetrated root **length**	PRL	2	7	1, 10, 21
Root **thickness**	RTHK	7	124	2, 3, 4, 6, 7, 8, 9, 10, 11, 13, 19, 21
Penetrated root **thickness**	PRTHK	3	37	1, 10, 21, 24
Root **branching** index	RBI	1	3	5
Root **growth rate** in depth	RGRD	1	14	20
Root **penetration** index	RPI	5	35	1, 10, 12, 14, 21, 24
Root **pulling force**	RPF	1	12	10, 21

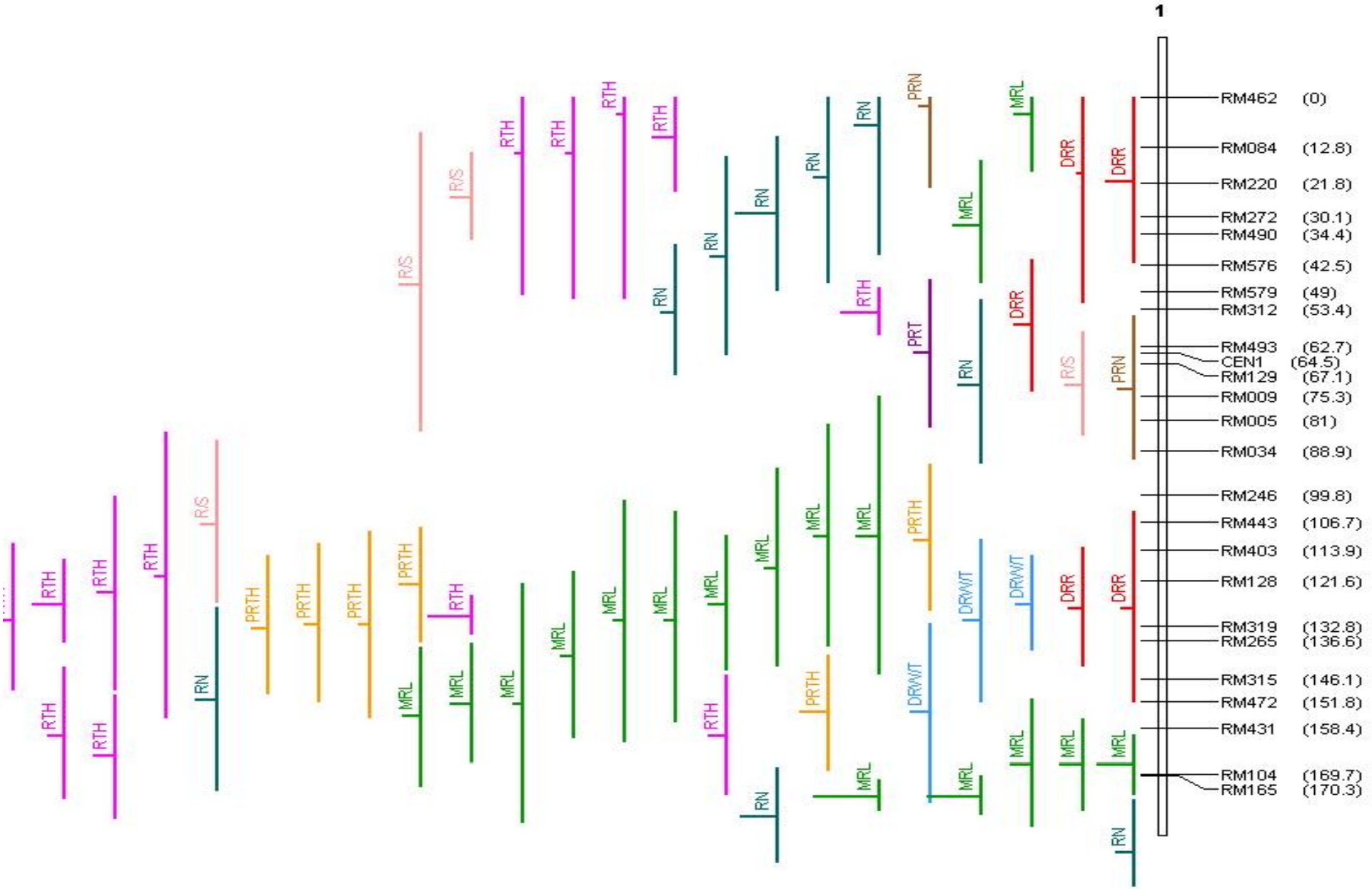
29 root traits mapped

Trait name	Symbol	Nbr populations	Nbr QTLs	Reference of the studies
Root **growth rate** in volume	RGRV	1	19	20
Root dry **weight**	RDW	6	40	3, 4, 5, 8, 10, 18, 19, 21
Root dry **weight** in the 00-30cm layer	RW0030	1	5	2
Root dry **weight** in the 30-60cm layer	RW3060	1	3	2
Root dry **weight** in the 60-90cm layer	RW6090	1	2	2
Deep root **weight**	DRW	5	43	3, 6, 7, 9, 13, 19
Root fresh **weight**	RFW	1	5	8
Penetrated root dry **weight**	PRDW	1	7	10, 21
Deep root ratio	DRR	3	31	6, 7, 20
Root to total biomass **ratio**	R/BIOM	1	16	9
Root to shoot **ratio**	R/S	4	32	2, 8, 13, 18
Deep root to shoot **ratio**	DR/S	2	7	3, 19
Root dry weight per tiller **ratio**	R/T	1	14	2
Deep root weight per tiller **ratio**	DR/T	5	23	2, 3, 6, 7, 19
Total		**12**	**689**	

36 descriptors for QTL info

Unique Identifier	716	**Sign. threshold 5% LOD**	2,9
QTL (or gene) name	qMRL3_1	**Sign. Threshold P**	
Trait name	Maximum root length	**Linkage group**	3
Symbol	MRL	**Left locus (or peak locus)**	**C316**
Measurement method	Ruler	**L Start P**	5 981 462
Sampling method	5 plants; 40 das	**L Stop P**	5 982 796
Unit	cm	**Right locus**	**C63**
QTL study	Xu et al (2004)	**R Start P**	8 389 519
Year	2001	**R Stop P**	83 91 001
Place	Huazhong, China	**LOD max**	5,2
Exp. Cond.	Greenhouse	**P max**	
Exp. design	RCBD; 4 reps	**Phen R2 (%)**	5,7
Population name	Zhenshan97/Minghui63	**QTL add. effect**	0,5
Cross type	indica/indica	**No allele subs.**	
Population type	RILs F10	**Origin allele additive effect**	Minghui63
Population size	240	**QTL dom. effect**	
Detection method	CIM	**Epitasis eval.**	
Software	QTL Cartographer	**Reference**	Drought28

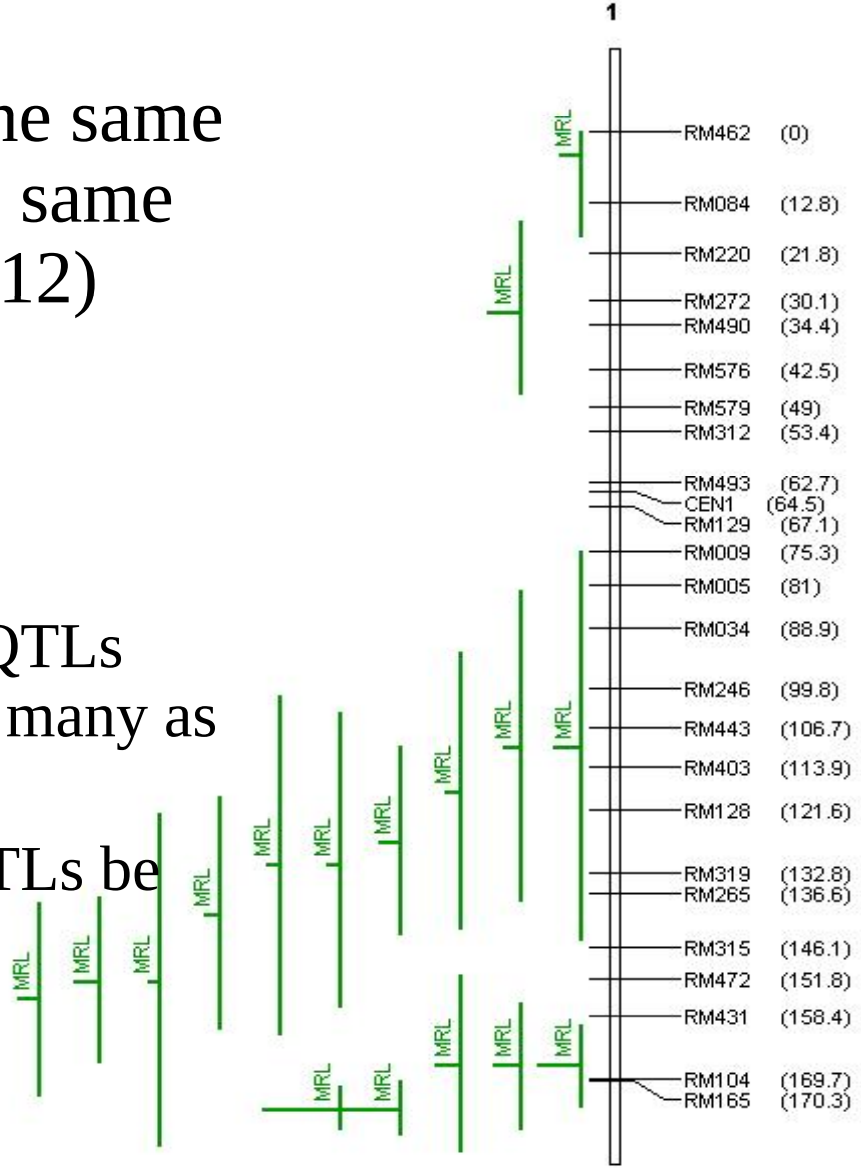
Extracting the meaningful information from the root QTL database



Extracting the meaningful information from the root QTL database

- Several (17) QTLs related to the same trait (MRL) are mapped on the same linkage group (1) in different (12) independent studies

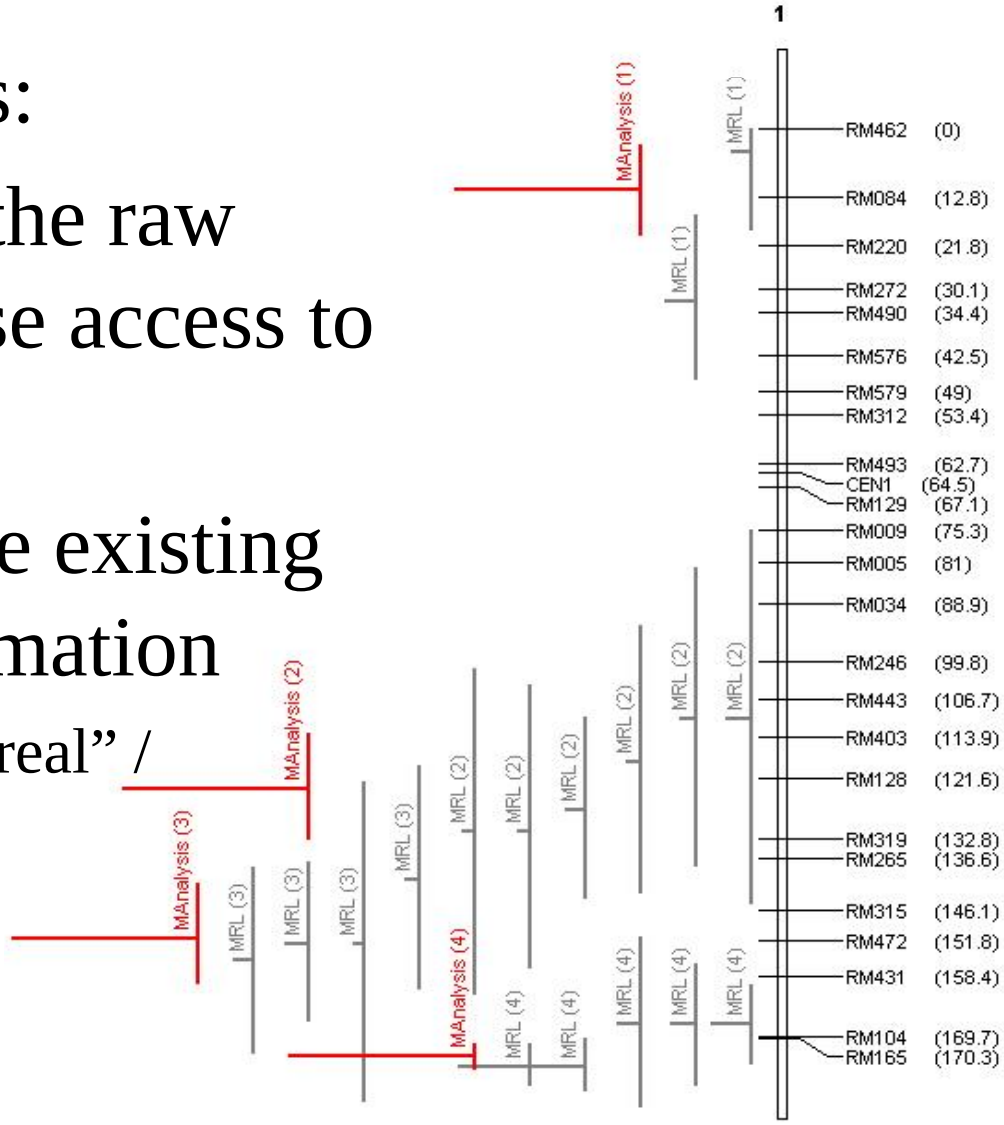
- Questions to address :
  - How many “real” QTLs do the QTLs detected represent ? 1, 2, 3 or as many as the number of QTLs detected ?
  - Can the position of the “real” QTLs be estimated ?



Extracting the meaningful information from the root QTL database

Two possible approaches:

- Pooled analysis using the raw datasets (which suppose access to raw data)
- Meta-analysis using the existing (published) QTL information
  - Determine the number of “real” / consensus QTLs
  - Estimate the position of each “real” QTL

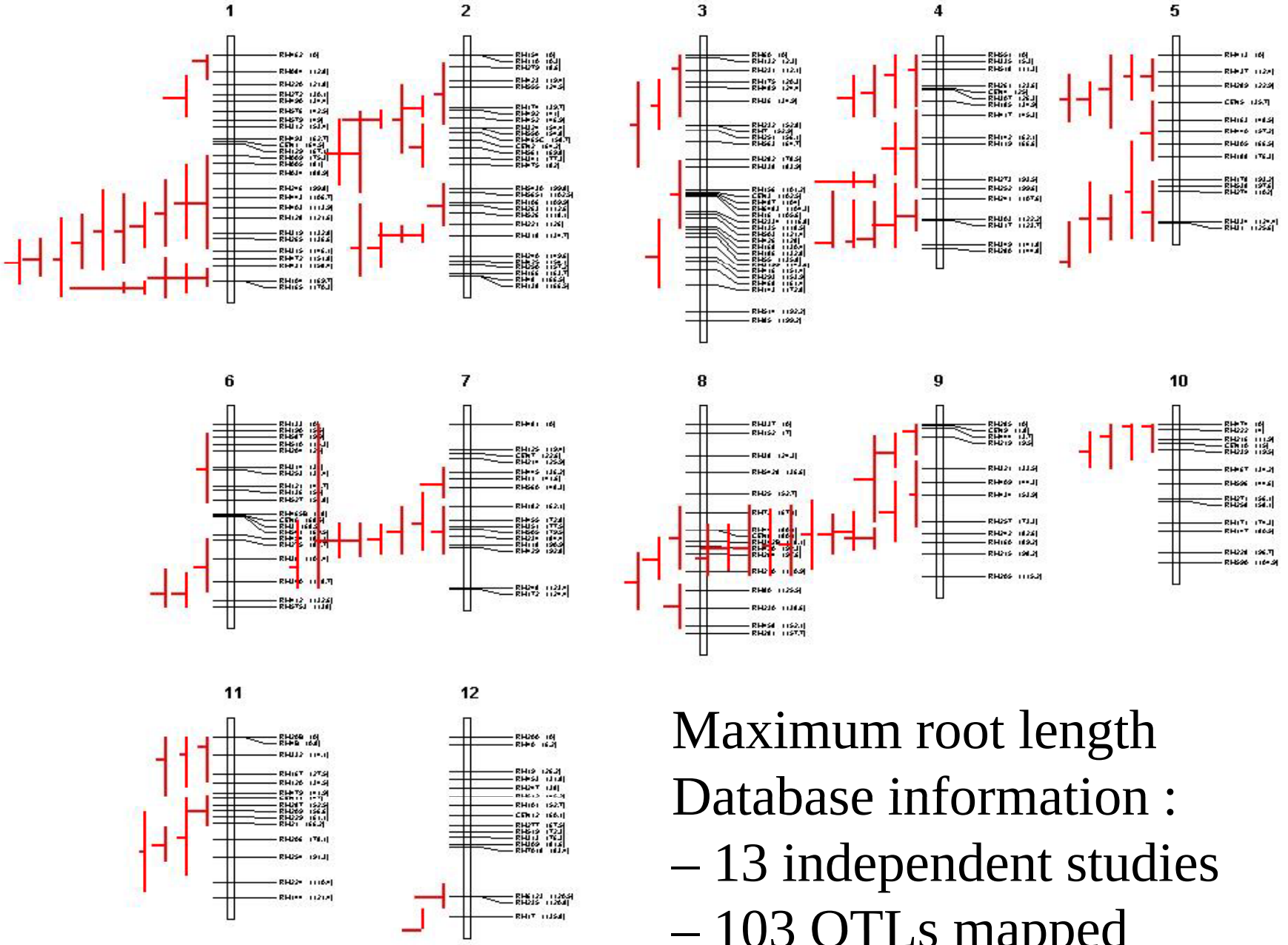


Meta-QTL analysis

- Projection of QTLs, from different studies, on a reference map
  - Determine the physical position of the markers flanking each QTL on the pseudochromosomes
  - Projection of physical position on a genetic reference map
- Estimation of a confidence interval of each QTL on the genetic reference map for, based on :
  - Population type : backcross, RIL, …
  - Population size
  - Proportion of the total phenotypic variation explained (R^2)
- Determination of the likely number of “real” QTLs for a given trait in a given chromosome using BioMercator software
  - Comparing different models for the “real” position of the n QTLs using an Akaike information criterion
  - Estimating the position of the “real” QTLs

BioMercator entree file

mapName	name	chr	trait	LOD	R2	SIM	Position	from	to
genetic_map	DRR1_1	1	DRR	2.5	16.4	N	21.39	0.00	42.94
genetic_map	DRR1_2	1	DRR	1.9	3.6	N	19.11	0.00	52.57
genetic_map	DRR1_3	1	DRR	5.6	10.6	N	57.81	41.14	74.48
genetic_map	DRR1_4	1	DRR	2.5	7.8	N	128.4	104.90	152.09
genetic_map	DRR1_5	1	DRR	2.9	8.1	N	128.4	113.62	143.37
genetic_map	DRR2_1	2	DRR	12.4	51.8	N	39.15	36.83	41.48
genetic_map	DRR2_2	2	DRR	6.1	15.8	N	39.15	31.53	46.78
genetic_map	DRR2_3	2	DRR	2.7	12.1	N	99.83	70.63	129.04
genetic_map	DRR2_4	2	DRR	6.0	20.3	N	122.04	113.34	130.74

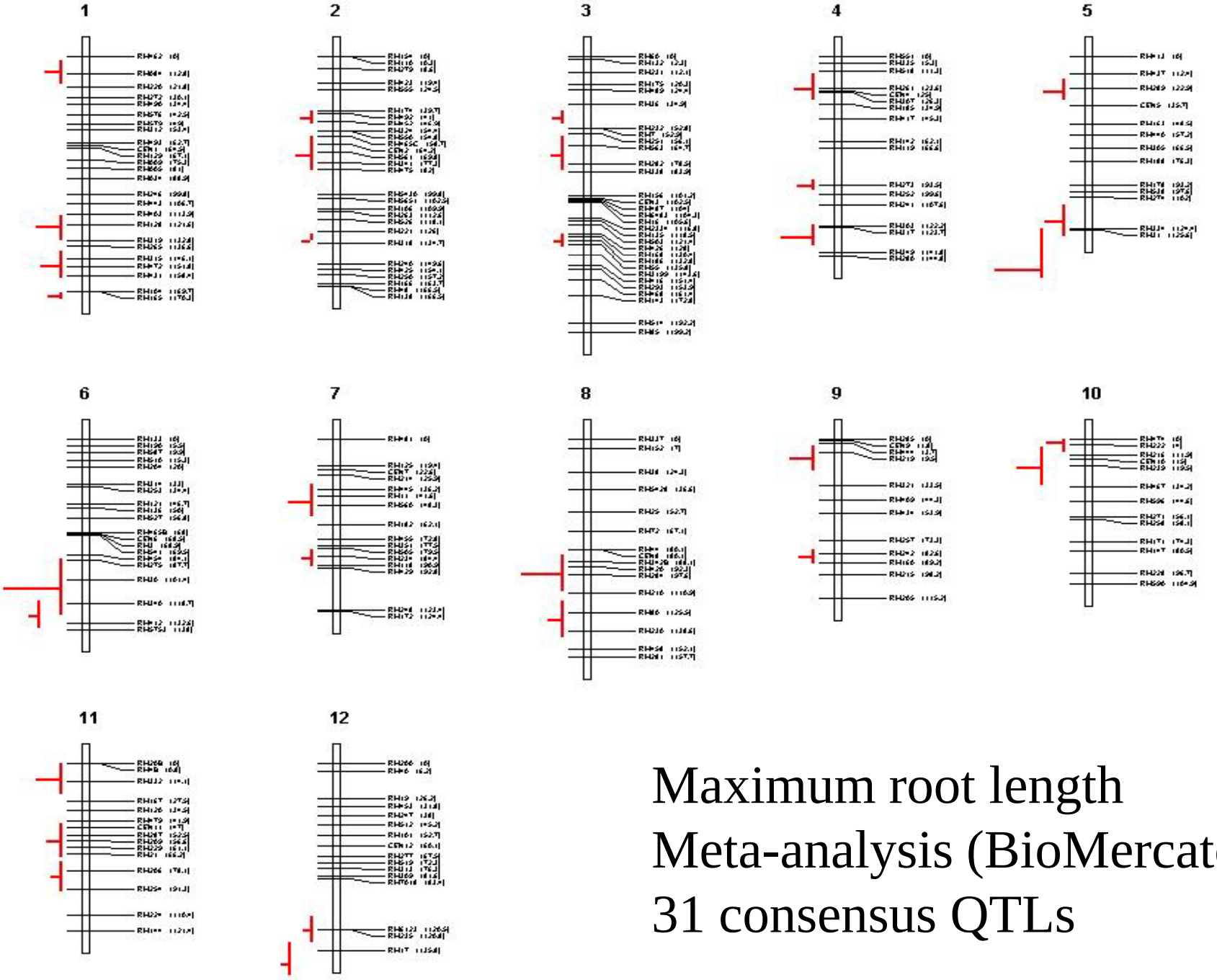


Maximum root length

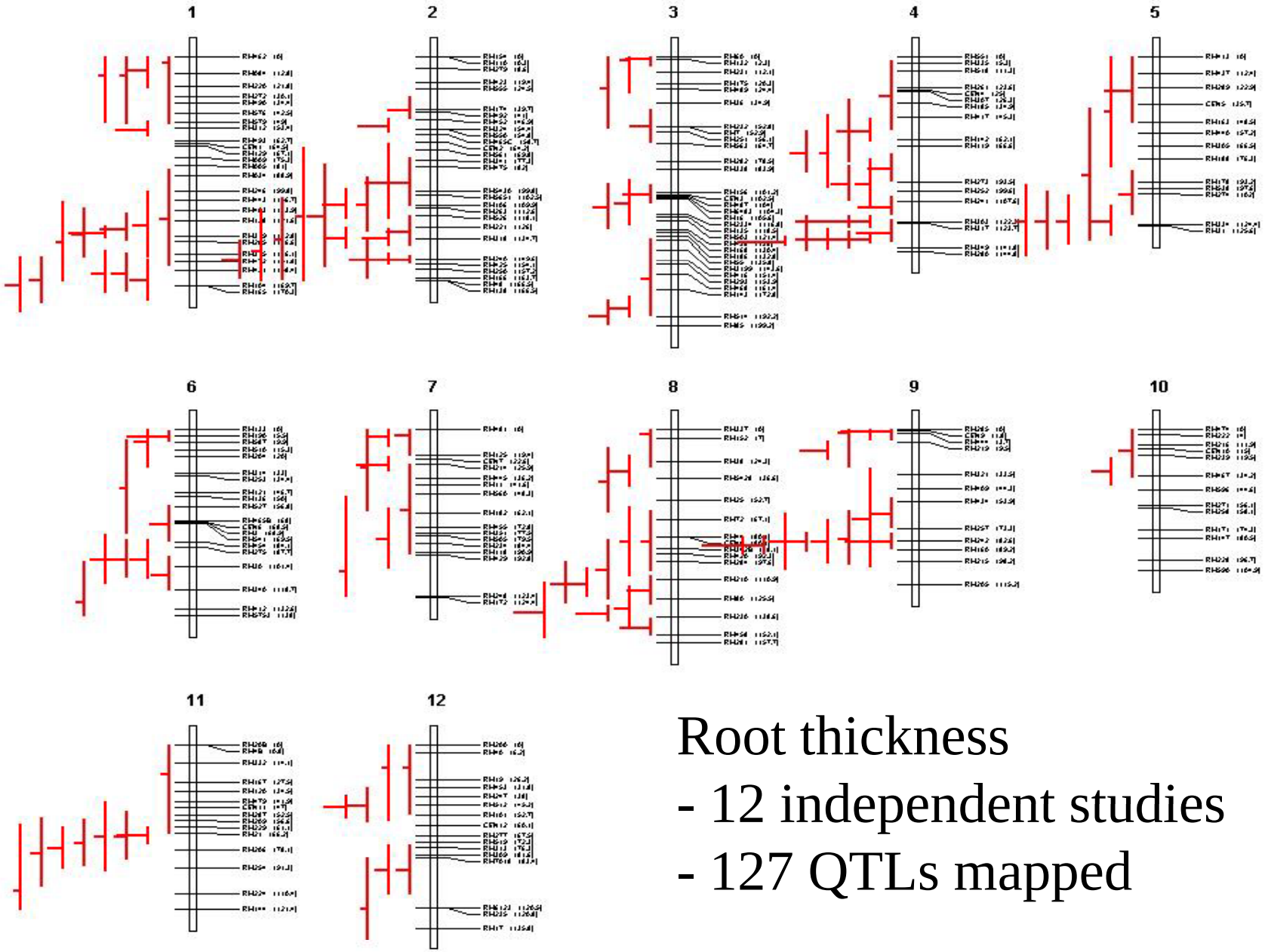
Database information :

– 13 independent studies

– 103 QTLs mapped

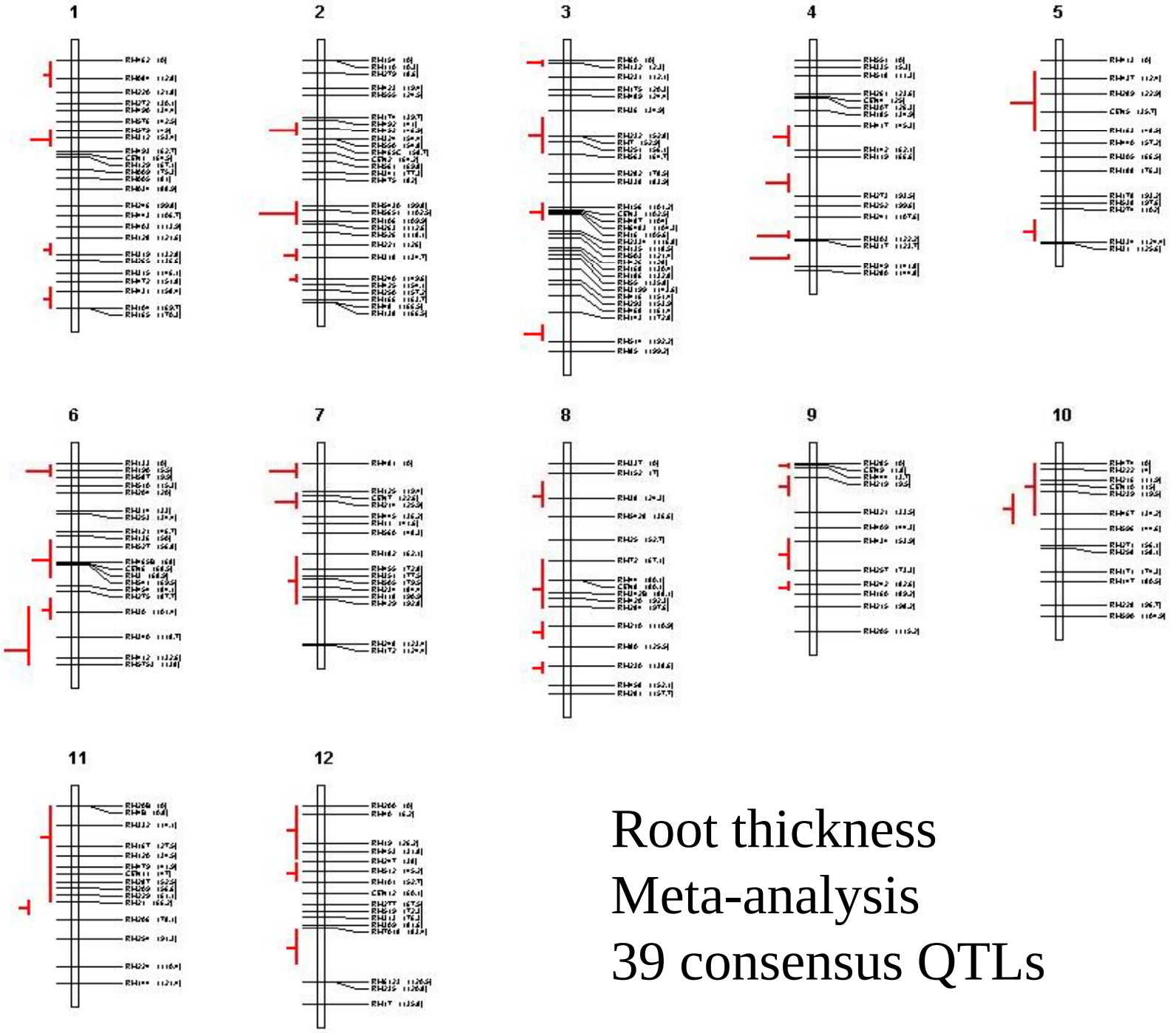


Maximum root length
Meta-analysis (BioMercator)
31 consensus QTLs



Root thickness

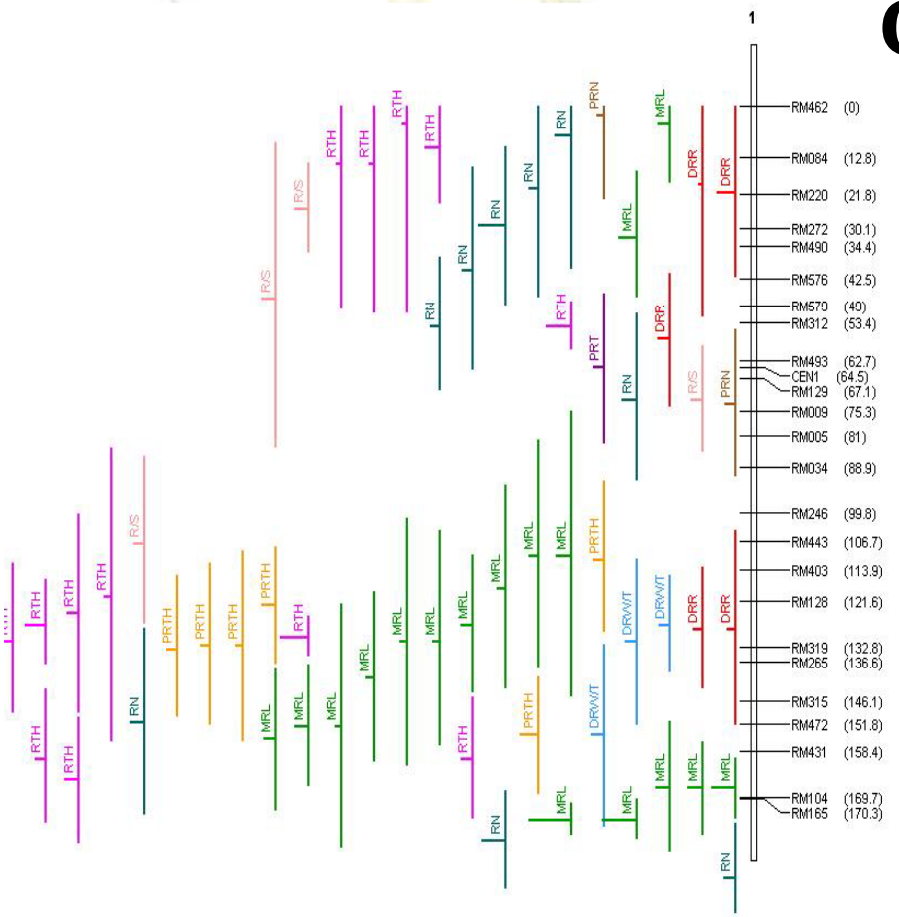
- 12 independent studies
- 127 QTLs mapped

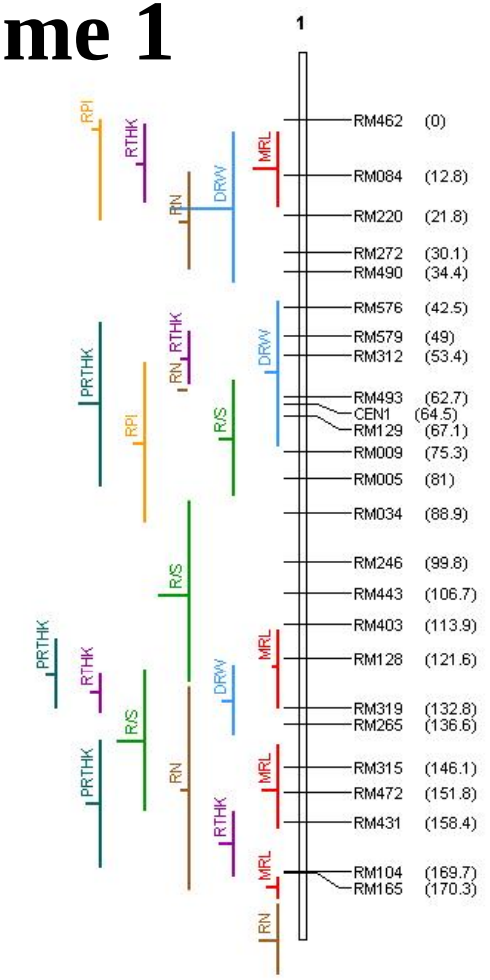


Root thickness
Meta-analysis
39 consensus QTLs

Overall view of root QTLs mapped

Chromosome 1





Root QTLs database

- 9 root traits
- **64 QTLs**

Meta analysis

- 9 root traits
- **20 QTLs**

Overall results of meta-analysis

Root trait	Chromosomes involved	Mapped QTLs	Consensus QTLs
Root thickness	12/12	127	39
Maximum root length	12/12	103	29
Root number	12/12	99	27
Deep root dry weight	9/12	33	20
Root / shoot ratio	11/12	33	11
Penetrated root number	11/12	27	16
Penetrated root thickness	10/12	37	17
Root penetration index	10/12	35	26
Total		494	145

Marker assisted selection strategy: targeting “Hot spots”

- Meta-analysis is not the panacea !
  - The number of QTLs involved in each root trait remains too high for MAS purpose
  - The accuracy of the number and the position of consensus QTLs depends upon the quality of the individual QTL mapping studies (genetic, phenotyping methods, QTL detection methods and software)
- Some chromosomal segments bear a high number of QTLs involved in root development
  - QTLs involved in one specific root trait in almost all genetic backgrounds and experimental conditions
  - QTLs involved in several root traits : MRL, RTHK, RN, …

“Hot spots”

- **C1** (30-40 Mb): MRL, RTHK, RN, RDW, R/S
- **C2** (25-35 Mb): MRL, RTHK, RN, DRDW, R/S RPI
- **C3** (0-5 Mb): RN, RTHK, RVol, RPI, RPF
- **C4** (25-35 Mb): MRL, RTHK, RVol, PRI, RPF
- **C6** (25-30 Mb): MRL, RTHK, RDW

Chr	d(Mb)	RN	DRN	LRN	PRN	SRL	MRL	LRL	PRL	PRTHK	RTHK	RVOL	RW0030	RW3060	RW6090	RFW	RDW	PRDW	DRW	DRR	R/BIOM	R/T	DR/T	R/S	DR/S	RBI	RGRD	RGRV	RPI	RPF	
1	0-5	2			1		1				4								1	2											11
1	5-10	2					1										1					1		1							6
1	10-15	1									1									1				1		1					5
1	15-20									1						1															2
1	20-25	1			1											1						1		1							5
1	25-30						2	1		1	1													1								6
1	30-35						4		1	4	5						1		1	2											18
1	35-40	1				2	4			1	5	2	1				2		1		1		2	1	1			2			26
1	40-44	2	2				3										1		2				1								11
2	0-5						1		1	1							1														4
2	5-10						1				1								3	2		1	1					1			10
2	10-15					2	2			1	1						1				4		3	1							15
2	15-20	1					2					1				1	1						2								8
2	20-25	1			2						4									1									1		9
2	25-30	2	1		4		3			1	6								1	1		1	1	2				1	5	2	31
2	30-35		1		3		2			4	1						2		3	2			1		1				3		23
2	35-36	1								1	4																		1		7
3	0-5	4	1		1		1			1	3	5					1		2		1	1		1			2	3	1	2	30
3	5-10			1			2				1					1															5
3	10-15	2						1									1												2	2	8
3	15-20	1			2						1								1	1				1					2		9
3	20-25						1				2						2												1		6
3	25-30				1				1										1	1							1				5
3	30-35	2					1				3											1		1							8
3	35-36				1																										1
4	0-5						1			2																					3
4	5-10	1					1							1			3			1			1	2	1						11
4	10-15	1					1				1								1				1								5
4	15-20				1		1				1			1						2	3						1		2		12
4	20-25						2				4								3	2		1	2								14
4	25-30	2					2			3	3	1						1		2				1				1	1		17
4	30-35	1		1	1		5				8	3						1					1	1				2	2	2	28
5	0-5						5	1			1						4				1								1		13
5	5-10										1	1								1			1		1						5
5	10-15						1																								1
5	15-20	1								1							1	1												2	6
5	20-25						1				2	1							1										1		6
5	25-30		1				4				4						1		1		2										13
6	0-5	1									2		1						1		1	1	1	1							9
6	5-10						1				1													1								3
6	10-15							1				1																				2
6	15-20	1								2	1						1							1								6
6	20-25	1		1	1					2							1										1				7
6	25-30						3	1			5						2		1		1					1			1		15
6	30-31																														0

“Hot spots”

- **C7** (10-15 Mb): RN, RTHK, RVol
- **C7** (25-30 Mb): MRL, RTHK, DRDW, R/S RPI
- **C9** (15-20 Mb): MRL, RTHK, RDW, R/S, RN
- **C11**(15-20 Mb): RTHK, RDW, PRI, RPF

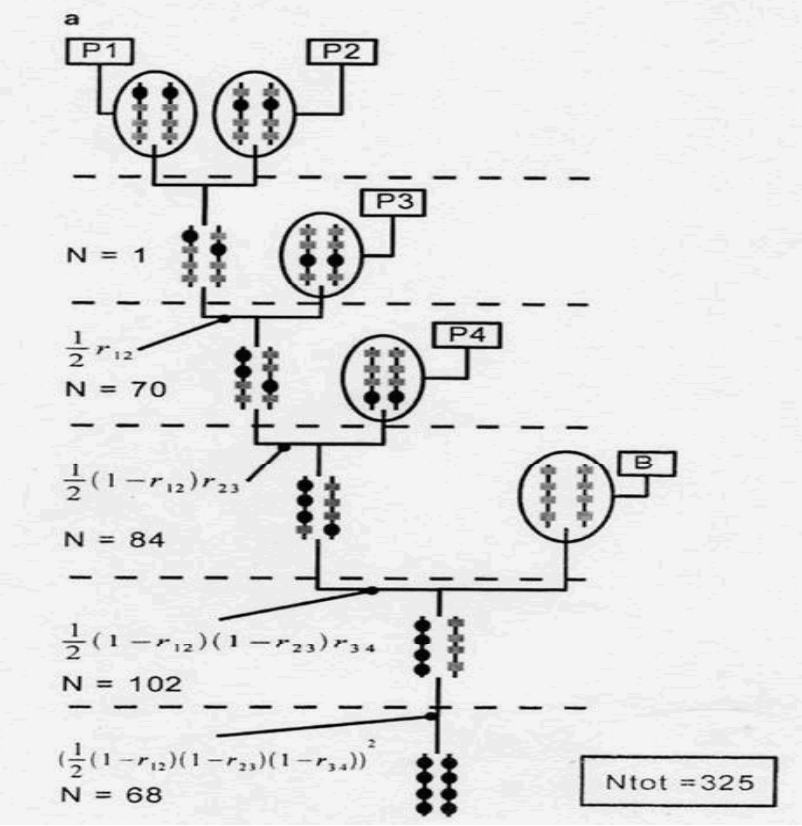
Chr	d(Mb)	RN	DRN	LRN	PRN	SRL	MRL	LRL	PRL	PRTHK	RTHK	RVOL	RW0030	RW3060	RW6090	RFW	RDW	PRDW	DRW	DRR	R/BIOM	R/T	DR/T	R/S	DR/S	RBI	RGRD	RGRV	RPI	RPF	
7	0-5	2								2	2								1			1									8
7	5-10																										3				3
7	10-15	4							1		2	4											1	1				2			15
7	15-20						1																								1
7	20-25				2	1	3				2	1							2					1							12
7	25-30						6			2	1		1				2		2	1		1	1	1	1			1	1		21
8	0-5				1						3			1					1										1	7
8	5-10				1																						1	1	1		4
8	10-15		1								2																		1		4
8	15-20						1					2							1									1	1		6
8	20-25	1					2				4								1					1							9
8	25-29		1				1				4											1		1							8
9	0-5				1		1			1	4									1											8
9	5-10	1									1									1		1		1			1				6
9	10-15						3										2		1					1							7
9	15-20	1	1				6			2	8						1	2	4	1			1	2	1						30
9	20-23	1	1			2	4												2		1				1						12
10	0-5		1				3																								4
10	5-10										1					1	1														3
10	10-15				1						1						1							2							5
10	15-20						1			1					1					1									1		5
10	20-23																2														2
11	0-5	1					3				1						2		1	2							1		1		12
11	5-10																						1								1
11	10-15																1									1		4			6
11	15-20	1							1		3		1		1		1		3	3				2			2		1	2	21
11	20-25	2			1		3				3									1	1		1						1		13
11	25-29								2		1						1							1							5
12	0-5	1									2																				3
12	5-10				1						2													1							4
12	10-15																														0
12	15-20									2								2				1					1		2		8
12	20-25									1	3																				4
12	25-30						2						1									1									4
12	30-34																														0

Strategy for using “hot spots”

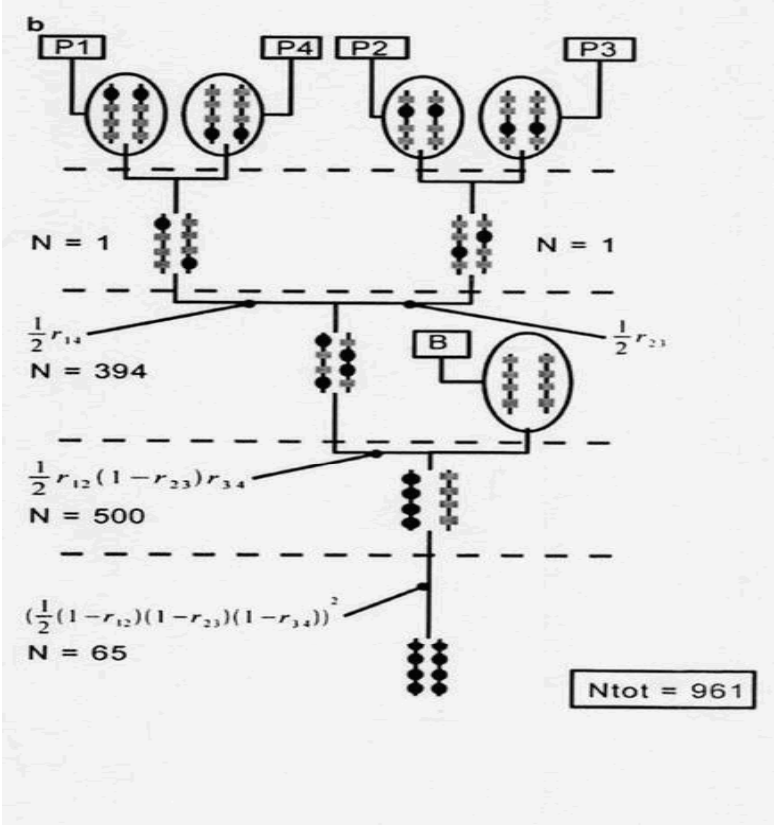
- Short term strategy: MAS for consensus QTLs
  - Mapping consensus QTLs for all root trait within each “hot spots”
  - MAS for 4-5 consensus QTLs
- Mid term strategy : fine mapping of consensus QTLs with following steps:
  - Mapping consensus QTLs for individual root traits within “hot spots”
    - QTLs involved in the constitutive pathway
    - QTLs involved in the adaptive pathway (RGR, R/S, RTHK, …)
  - Fine mapping of major consensus QTLs
  - Looking for co-localisation of candidate genes and finely mapped QTLs

MAS strategies

Backcross : Introgression of favorable alleles coming from a line with limited agronomic value into a good genetic background



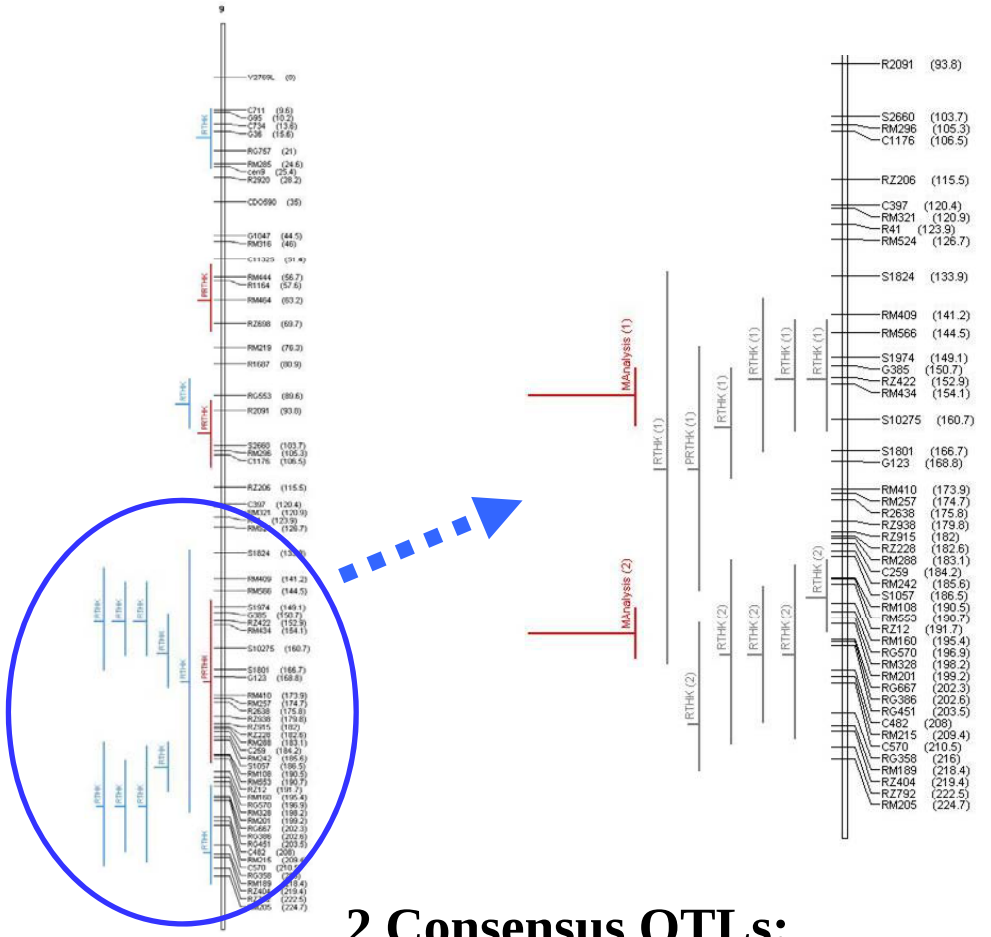
Genotype building : by pyramiding several alleles from different parents into elite genotype (Servin et al, 2004).



Fine mapping consensus QTLs on chromosome 9 “hot spot”

Maximum root length

- 12 QTLs from 7 studies and 12 independent conditions
- 2 consensus QTLs with:
  - Improved precision in t QTL location
  - Smaller confidence interval

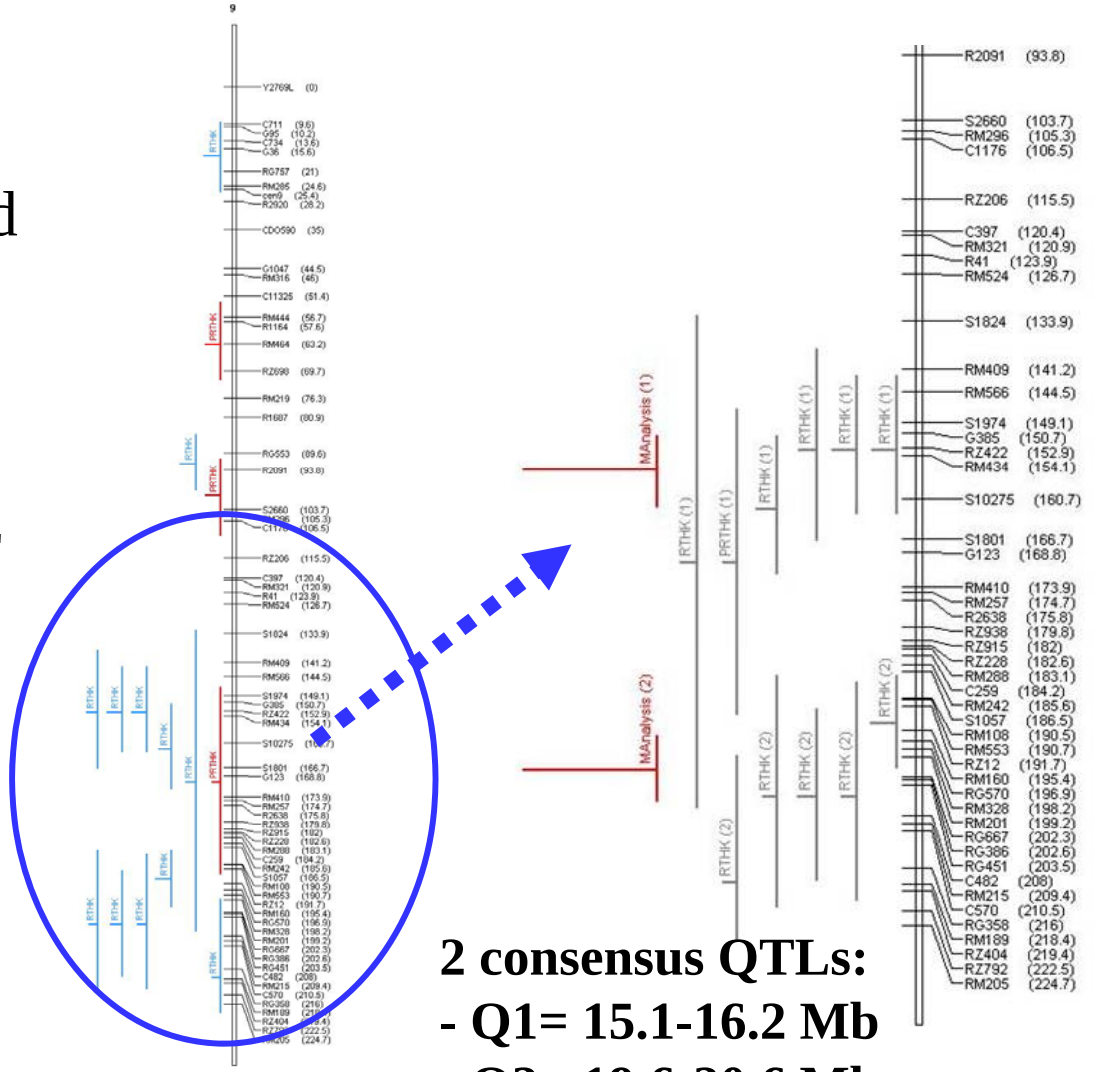


2 Consensus QTLs:
- Q1= 15.1-16.2 Mb
- Q2= 19.6-20.6 Mb

Fine mapping consensus QTLs on chromosome 9 “hot spot”

Root thickness

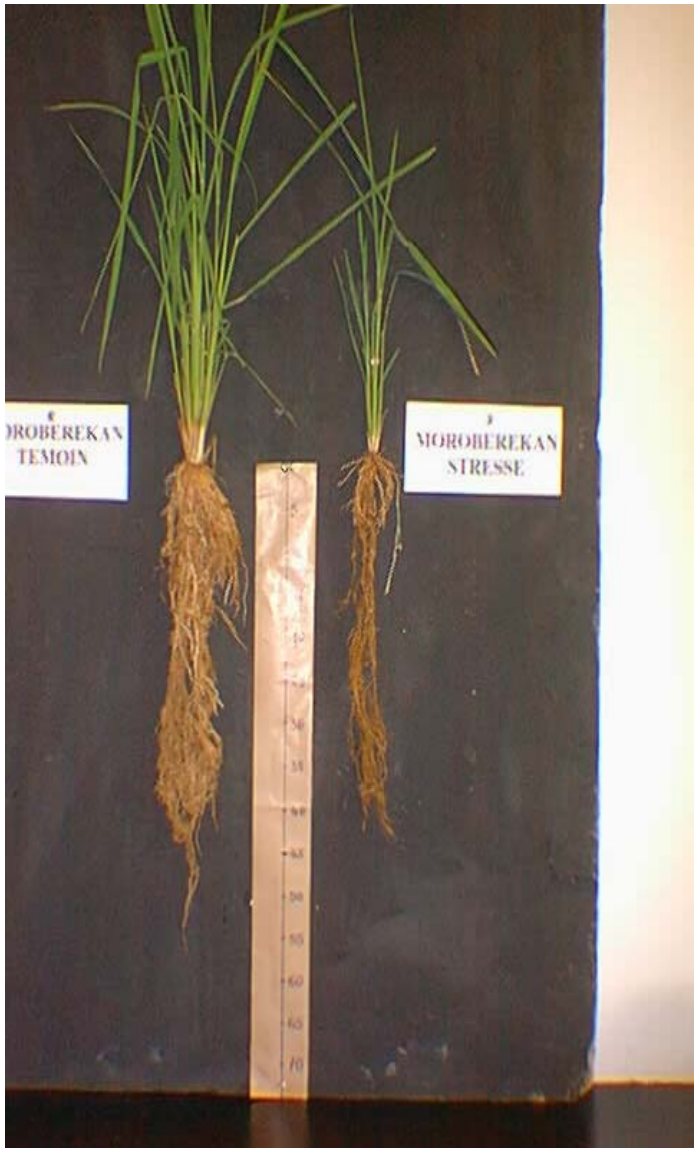
- 15 QTLs from 6 studies and 15 independent conditions
- 2 consensus QTLs with:
  - Improved precision in the QTL location
  - Smaller confidence interval
- Issue to be addressed:
  - How accurate are the meta-QTLs?
  - To be checked with cloned QTLs



2 consensus QTLs:
- Q1= 15.1-16.2 Mb
- Q2= 19.6-20.6 Mb

Beyond MAS for root QTL

- Is genetic potential the first limiting factor for root development in target environments ?
  - Requires strong interaction with agronomists
- Is there enough genetic diversity within *O. sativa* ?
  - Need for a quantitative (modelling) approach of the contribution of root development to drought avoidance
- Can genetic engineering enhance root development (optimizing the expression of native genes or introducing new mechanisms) ?
  - Need for a good understanding of genes and genes networks involved in root development



Prospects

- Link molecular genetics to molecular biology
  - Isolation of QTLs involved in root development
  - Co-localisation of root-specific architectural genes / transcripts with constitutive / adaptive QTLs
  - Isolate genes through mutant screenings
  - ➢ Need for robust root architecture phenotyping procedures

- Decipher the molecular control of root development and plasticity
  - Test conservation of function with focus on receptors and transcription factors (QHB, PLT,SCR, SHR,, NAC1..) : *in silico* prediction of orthologs, expression profiling, reverse genetics approach
  - Search upstream regulators including miRNA,
  - Search downstream effectors
  - Hormonal control and stress response

Thank you for your attention

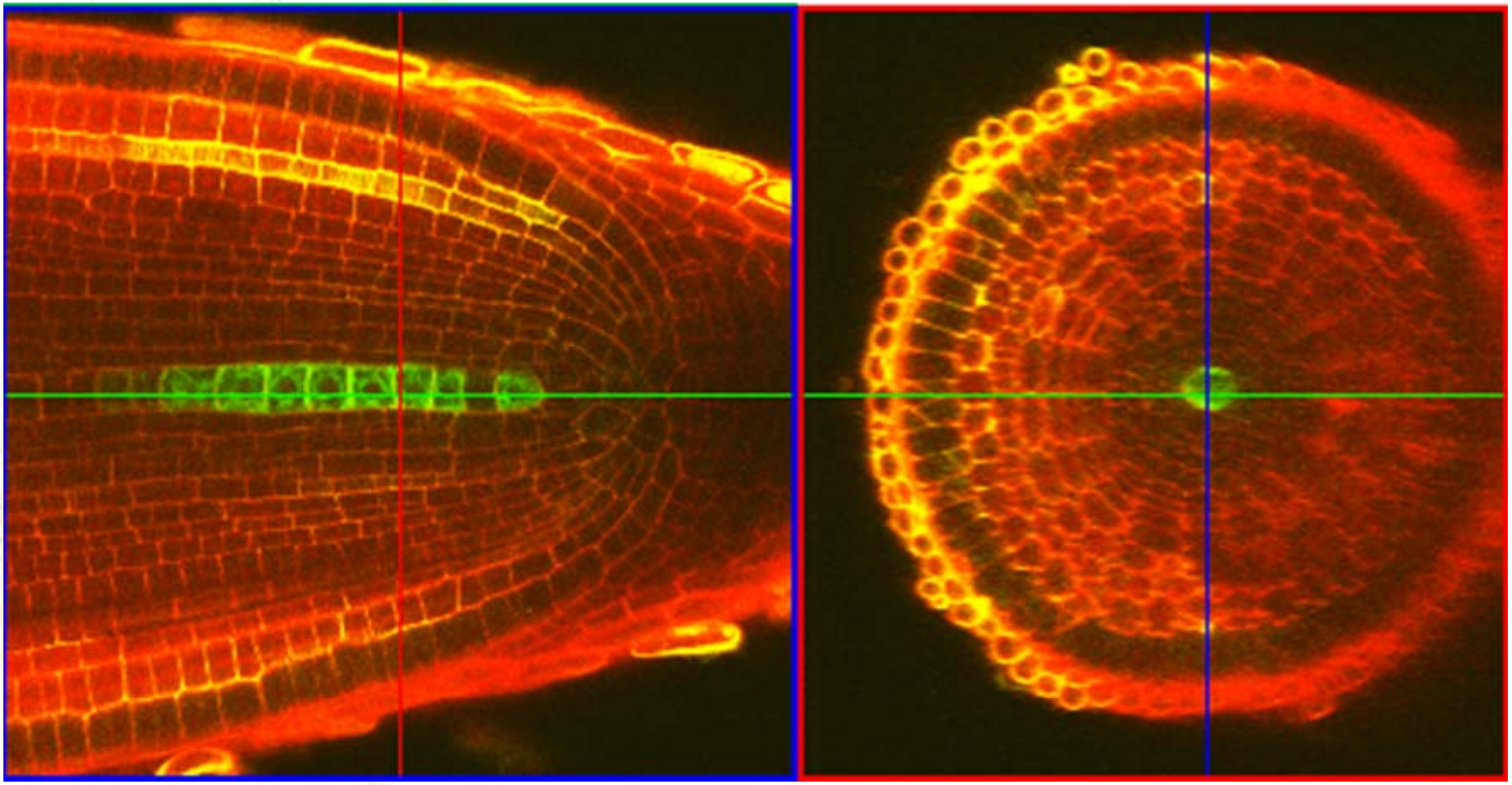


Illustration in a metaxylem-specific GAL4:GFP ET line

(E. Guiderdoni, C. Perin)

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Rice root genetic architecture: meta-analysis from a QTL database

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The genetic architecture of rice root system has been extensively studied by teams interested in improving drought resistance of rice. Many papers have been published in the last 10 years on QTL detection for root parameters in several populations. These combined data could be extremely helpful for candidate gene identification or QTL cloning provided an easy access to the whole data is possible.
Our objective was to store the relevant data in an easy to query database and synthetize these results in a way useful for geneticists interested in rice root system through a metaQTL analysis.
We extracted from 24 papers all relevant information on QTLs controlling root parameters. A database of 689 QTLs for 29 traits detected in 12 populations was constituted. The physical position on the pseudochromosomes of the markers flanking the QTLs was determined. For this purpose, Gramene data or sequence information were used when available. Otherwise, as in the case of AFLPs, the nearest marker with a known sequence was used. An overview of the number of QTLs in segments of 5 Mb covering the whole genome is presented. Areas with a large density of QTLs for any given traits were further explored through a meta-QTL analysis approach using the software package BioMercator. The method allowed us to determine the likely number of true QTLs in these areas using an Akaike information criterion, and estimate their position . The consensus QTL confidence interval was generally reduced.
Existing bioinformatics tools such as OrygenesDB (http://orygenesdb.cirad.fr/) can be used to list the genes underlying consensus QTL confidence intervals.
The QTL basic information will be soon available to the scientific community in Tropgene database. (http://tropgenedb.cirad.fr/). Complex queries on the experimental conditions in which QTL were detected will be possible. Beside root data, the database includes similar data on other drought avoidance or drought tolerance traits.